



Ain Shams University

Faculty of Women for Arts, Science and Education

Growth promoting and phytopathogen antagonistic effects of rhizobacteria: Application on *Phaseolus vulgaris* L. in Gaza Strip

A thesis

Submitted in Partial Fulfillment of The Requirements for the Doctor of Philosophy Degree in Science (Microbiology)

By

Niddal Saleem Salman Abu Hujier

M.Sc. Biological Sciences/Medical Technology

Under Supervision

Prof. Dr. Mona Ishak Fahd

Prof. of Microbiology,
Botany Department,
Faculty of Women for Arts, Science
and Education, Ain Shams University

Prof. Dr. Fadel Akram Sharif

Prof. of Molecular Biology,
Medical Technology Department,
Faculty of Health Science,
Islamic University of Gaza

Dr. Shima Mohamed Abdelsalam

Lecturer of Microbiology,
Botany Department
Faculty of Women for Arts, Science
and Education, Ain Shams University

Ain Shams University
Faculty of women for Arts, Science and Education
Botany department
(2019)

PhD Thesis

Name: Niddal Saleem Salman Abu Hujier.

Title: Growth promoting and phytopathogen antagonistic effects of rhizobacteria: Application on *Phaseolus vulgaris* in Gaza Strip.

Scientific degree: Philosophy Doctor of Science (Microbiology).

Department: Botany.

Faculty: Faculty of Women for Arts, Science and Education.

University: Ain Shams University.

***This thesis has not been previously submitted for any degree at
this or any other university***

Signature

Niddal Saleem Salman Abu Hujier

Supervisors

Prof. Dr. Mona Ishak Fahd

Prof. of Microbiology, Botany Department,
Faculty of Women for Arts, Science and Education,
Ain Shams University

Prof. Dr. Fadel Akram Sharif

Prof. of Molecular Biology, Medical Technology Department,
faculty of Health Science,
Islamic University of Gaza

Dr. Shimaa Mohamad Abdelsalam

Lecturer of Microbiology, Botany Department
Faculty of Women for Arts, Science and Education
Ain Shams University

Approval Sheet

Approved by	Signature
Prof. Dr. Mona Ishak Fahd Prof. of Microbiology, Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University	
Prof. Dr. Fadel Akram Sharif Prof. of Molecular Biology, Medical Technology Department, faculty of Health Science, Islamic University of Gaza	
Dr. Shimaa Mohamad Abdelsalam Lecturer of Microbiology, Botany Department Faculty of Women for Arts, Science and Education Ain Shams University	

Acknowledgements

My thanks go to **Prof. Mona Ishak Fahd**, my great supervisor who has seen me through this whole process, for her positiveness, intelligence, love of excellence, love of healing, very nice comments, and discussion.

To **Prof. Fadel Akram Sharif**, without his efforts and creative ideas any of this would be possible, who walked so much of this journey with me, I also offer from my heart thanks.

I have been very well supported by **Dr. Shimaa Mohamad Abdelsalam**, who let me know she believed in the value of my work, and was always willing to lend practical support.

I have felt supported by **University College of Science and Technology**, Khan Younis. I am very Thankful for their help.

My **mother, father** who deserves special thanks, **sister** and **brothers** contributed greatly on this. I thank them for always being at my side. I am so proud of them.

I would like to thank my **beloved wife** for moral help.

I would like to extend my sincere thanks to the staff molecular lab in Islamic University of Gaza.

I would like to show my greatest appreciation to Ministry of Agriculture in Gaza for their support and help.

Niddal Saleem Salman Abu Hujier

Contents

Titles	Pages
List of tables	
List of figures	
List of abbreviations	
Abstract	
Introduction	1
Objectives and Plane of work.....	5
Literature Review	6
1. Introduction.....	6
2. PGPR and root colonization.....	7
2.1. PGPR.....	7
2.2. The process of root colonization.....	7
2.3. PGPR colonization traits.....	8
3. Role of PGPR for sustainable agriculture.....	8
3.1. Biofertilizers.....	9
4. Role of PGPR in plant disease suppression.....	17
4.1. Chitinase production.....	17
4.2. HCN Production.....	19
4.3. Production of antagonistic substances.....	19
4.4. Induced systemic resistance (ISR)	20
4.5. Common <i>P. vulgaris</i> L. disease causing agents.....	23
4.6. Biocontrol of <i>F. oxysporum</i>	28
4.7. Biocontrol of <i>M. phaseolina</i>	28
5. Consortia vs. single application of biocontrol PGPR.....	29
Materials and Methods	31
1. Bioprospecting for rhizobia.....	31

2. Isolation of plant growth promoting rhizobacteria (PGPR) strains.....	32
3. Detection of plant growth promotion traits of rhizobacterial isolated	33
3.1. Quantitative determination of IAA production.....	33
3.2. Qualitative determination of hydrogen cyanide (HCN) production.....	34
3.3. Qualitative determination of siderophore production.....	34
3.4. Qualitative determination of Zinc solubilization activity.....	35
3.5. Qualitative determination of chitin solubilization activity.....	35
3.6. Qualitative determination of inorganic phosphate solubilization activity.....	36
3.7. Qualitative determination of ACC deaminase activity.....	36
3.8. Quantitative determination of phytase production.....	37
4. Biochemical identification tests of PGPR isolates.....	38
5. Isolation of phytopathogens.....	38
5.1. Isolation of <i>F. oxysporum</i>	38
5.2. Isolation of <i>M. phaseolina</i>	39
6. Detecting the antagonistic activities of the isolates against phytopathogenic fungi <i>in-vitro</i>	40
7. Detection of compatibility between selected isolates.....	41
8. Selection of isolates for subsequent pot experiment.....	41
9. Molecular identification.....	41
10. Plant inoculation assay.....	42
11. Analysis of soil used in pot experiments.....	43
12. Physical and chemical analysis of water used in pot experiments ...	43
13. Experimental measurements.....	45

13.1. Root length.....	45
13.2. Shoot height.....	46
13.3. Number of leaves per plant.....	46
13.4. Leaf surface area.....	46
13.5. Branch number.....	46
13.6. Stem diameter.....	46
13.7. Time of flower appearance.....	46
13.8. Fresh weight.....	46
13.9. Dry weight.....	47
13.10. Appearance of diseases during growth period.....	47
13.11. Measurement of nitrogen percentage.....	47
14. Biocontrol of phytopathogens.....	47
14.1. Inoculation of <i>P. vulgaris</i> L. seeds by bacterial isolates... ..	47
14.2. Inoculation with the fungal pathogens.....	48
14.3. Disease Evaluation.....	49
15. Measurement of plant growth parameters	49
16. Statistical analysis.....	49
Results	51
1. Physiological PGP traits of isolated strains.....	51
1.1. Quantitative determination of IAA.....	51
1.2. Qualitative production of HCN.....	52
1.3. Qualitative production of siderophore.....	53
1.4. Qualitative determination of zinc solubilization activity.....	53
1.5. Qualitative determination of chitin solubilization activity.....	54
1.6. Qualitative determination of inorganic phosphate solubilization activity.....	54
1.7. Qualitative determination of ACC deaminase activity.....	55

1.8. Quantitative determination of phytase production.....	58
2. Preliminary screen according to PGP trait results.....	58
3. Biochemical identification tests of PGPR isolates.....	59
4. Antagonistic activities of the isolates against phytopathogenic fungi.....	60
5. Secondary screening of isolates upon plant growth promotion and biocontrol action.....	63
6. Compatibility tests between selected isolates	64
7. Final selection and establishment of bacterial consortia for pot experiments.....	64
8. Results of PCR amplification of partial 16SDNA gene.....	65
9. Molecular identification of selected PGPR by 16s rDNA sequencing	65
10. Chemical and physical properties of soil used in pot experiment....	70
11. Evaluation of Plant growth promoting activities of the selected PGPR strains.....	71
11.1. Pot experiments in non-sterile soil.....	71
11.2. Pot experiments in sterile soil.....	93
12. Effects of PGPR isolates against phytopathogenic fungi.....	113
12.1. Seedlings infected with <i>F. oxysporum</i>	113
12.2. Seedlings infected with <i>M. phaseolina</i>	125
12.3. Seedlings infected with <i>F. oxysporum</i> and <i>M. phaseolina</i> mixture.....	135
12.4. Treatments induced plant growth parameters to significantly equivalent level of healthy control.....	149

12.5. Synopsis of PGPR isolates performance regarding biocontrol and growth promotion.....	152
Discussion	153
Summary	168
Conclusion	172
References	173
Appendices	221
11- Arabic summary	1-4

List of tables

Title	Pages
Table (1): Chemical properties of Beit lahia soil.	32
Table (2): Physical and chemical analysis of water used in pot experiments.	44
Table (3): Bacterial treatments used in pot experiment studies	44
Table (4): Plant growth-promoting traits of G ⁻ bacterial isolates.	56
Table (5): Plant growth-promoting traits of G ⁺ bacterial isolates.	57
Table (6): Biochemical identification reactions of preliminary selected isolates.	61
Table (7): Closest possible genera of isolated rhizobacteria.	62
Table (8): <i>In vitro</i> screening of G ⁻ PGPR strains against <i>F. oxysporum</i> and <i>Macrophomina phaseolina</i> .	63
Table (9): <i>In vitro</i> screening of G ⁺ PGPR strains against <i>F. oxysporum</i> and <i>Macrophomina phaseolina</i> .	63
Table (10): Compatibility test between the seven selected isolates.	64
Table (11): Biochemical and antagonistic characteristics of final screened isolates.	65
Table (12): Identity of isolates by partial 16S- rDNA sequence analysis.	70
Table (13): Chemical and physical properties of soil used in pot experiment.	71
Table (14): Effect of bacterial treatments on root length and shoot height in cm of <i>P. vulgaris</i> L.	74

Table (15): Effect of bacterial treatments on leaves number and leaves surface area of <i>P. vulgaris</i> L.	75
Table (16): Effects of different bacterial treatments on branch number plant ⁻¹ and stem diameter of <i>P. vulgaris</i> L.	79
Table (17): Effects of different bacterial isolates on time of flower appearance.	81
Table (18): Effect of bacterial treatments on fresh weight, dry weight, and nitrogen concentration of <i>P. vulgaris</i> L.	84
Table (19): Diseases that appeared during growth of <i>P. vulgaris</i> L. seedlings.	87
Table (20): Synopsis of PGPR isolates (alone or mixture) that afforded significant increase in different plant growth parameters of <i>P. vulgaris</i> L. compared to control.	89
Table (21). Growth promotion effects of 19 treatments on <i>P. vulgaris</i> L. plants in greenhouse pot experiments, with the four consortia having the highest (GSF) and (FIT ₃) percentages highlighted with shading.	91
Table (22): Pearson correlation between vegetative induced variables.	92
Table (23): Effect of bacterial treatments on root length and shoot height of <i>P. vulgaris</i> L. in sterile soil.	95
Table (24): Effect of different bacterial treatments on number and surface area of seedling leaves.	98
Table (25): Effects of bacterial treatments on branch number and stem diameter of <i>P. vulgaris</i> L.	100
Table (26): Effects of bacterial treatments on time of flowering of <i>P. vulgaris</i> L. in sterile soil.	103
Table (27): Effects of bacterial isolates on fresh and dry weight of <i>P.</i>	105

<i>vulgaris</i> L. in sterile soil.	
Table (28): Effects of bacterial isolates on nitrogen contents of <i>P. vulgaris</i> L. in sterile soil.	107
Table (29): Disease occurrence during growth of <i>P. vulgaris</i> L. in sterile soil.	108
Table (30): Synopsis of PGPR isolates (alone or mixture) that afforded significant increase in different plant growth parameters of <i>P. vulgaris</i> L. compared to control in sterile soil.	110
Table (31). Growth promotion in sterile soil of 19 treatments on <i>P. vulgaris</i> L. plants in greenhouse pot experiments, with the five consortia having the highest (GSF) and (FIT ₃) percentages indicated with shading.	112
Table (32): Effects of selected PGPR isolates on resistance pattern of <i>P. vulgaris</i> L. treated with <i>F. oxysporum</i> .	115
Table (33): Fresh and dry weights of <i>F. oxysporum</i> infected <i>P. vulgaris</i> L. plants under selected PGPR isolates inoculation and controls.	117
Table (34): Leaves number plant ⁻¹ and shoot length of <i>F. oxysporum</i> infected <i>P. vulgaris</i> plants under selected PGPR treatments inoculation and controls.	119
Table (35): Pods number plant ⁻¹ , pods fresh weight plant ⁻¹ , and pod dry weight plant ⁻¹ of <i>F. oxysporum</i> infected <i>P. vulgaris</i> L. plants under selected PGPR isolates inoculation and controls.	122
Table (36); Growth promotion effects of 19 treatments on <i>P. vulgaris</i> L. plants infected with <i>F. oxysporum</i> in greenhouse pot experiments.	124
Table (37): Effect of rhizobacterial isolates on resistance pattern of <i>P. vulgaris</i> L. plants treated with <i>M. phaseolina</i> .	126
Table (38): Fresh weight, dry weight, leaves number plant ⁻¹ and shoot	132

length of <i>M. phaseolina</i> infected <i>P. vulgaris</i> L. plants under selected PGPR treatments inoculation and controls.	
Table (39): Pods number plant ⁻¹ , pods fresh weight plant ⁻¹ , and pod dry weight plant ⁻¹ of <i>M. phaseolina</i> infected <i>P. vulgaris</i> L. plants under selected PGPR isolates inoculation and controls.	134
Table (40): Growth promotion effects of 19 treatments on <i>P. vulgaris</i> L. plants infected with <i>M. phaseolina</i> in greenhouse pot experiments.	136
Table (41): Effect of selected PGPR isolates on resistance pattern of treated seedlings with <i>F. oxysporum</i> and <i>M. phaseolina</i> mixture.	137
Table (42): Fresh and dry weights of <i>F. oxysporum</i> and <i>M. phaseolina</i> mixed infected <i>P. vulgaris</i> L. seedlings under selected PGPR isolates inoculation and controls.	140
Table (43): Leaves number plant ⁻¹ and shoot length of <i>F. oxysporum</i> and <i>M. phaseolina</i> mixed infected <i>P. vulgaris</i> L. seedlings under selected PGPR isolates inoculation and controls.	142
Table (44): Pods number plant ⁻¹ , pods fresh weight plant ⁻¹ , and pod dry weight plant ⁻¹ of <i>F. oxysporum</i> and <i>M. phaseolina</i> mixed infected <i>P. vulgaris</i> L. plants under selected PGPR consortia inoculation and control.	145
Table (45): Growth promotion effects of treatments on <i>P. vulgaris</i> L. plants infected with <i>F. oxysporum</i> and <i>M. phaseolina</i> mixture.	148
Table (46): Treatments that afforded plant growth promotion to significantly equivalent level of healthy control	150